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Gas chromatographic determination of ethosuximide and phensuximide in plasma and urine of man

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Ethosuximide and phensuximide in plasma and urine can be assayed to a concentration of 2·5 μg ml^{-1} by a rapid and specific gas chromatographic assay. The method can be used to monitor plasma concentrations in patients with petit mal treated with these drugs alone or in combination with other antiepileptic drugs. The steady-state plasma concentrations of ethosuximide in children given 10–45 mg kg^{-1} day^{-1} varied between 10 and 150 μg ml^{-1} indicating marked interindividual differences in pharmacokinetics.

Epileptics are usually treated with a combination of drugs that hampers the evaluation of individual drug effects. Phenytoin concentrations in plasma have been successfully monitored, the relation between the steady-state plasma concentration and the clinical effects is known (Buchtal, Svensmark & Schiller, 1960; Lund, Lunde & others, 1971).

Ethosuximide and phensuximide have been extensively used in the treatment of petit mal epilepsy for over 15 years but few reports are available on their pharmacokinetics in patients.

An unspecific spectrophotometric method for the determination of ethosuximide has been described by Hansen (1963) and more recently Buchanan, Fernandez & Kinkel (1969) reported a gas chromatographic method and in single dose studies found a plasma half life of the drug of approximately 30 h in five children. Meijer (1971) described gas chromatographic systems for the separation of various antiepileptic drugs including ethosuximide but reported no quantitative data. No specific method seems to be available for phensuximide.

We describe a gas chromatographic method for ethosuximide and phensuximide that is selective in relation to other antiepileptic drugs and simple enough for routine clinical use. We also report preliminary investigations of plasma concentrations of ethosuximide in children with petit mal epilepsy.

MATERIALS AND METHODS

Ethosuximide, (3-ethyl-3-methylsuccinimide, Suxinutin, Zarontin, (Parke Davis, Detroit); phensuximide, (1-methyl-3-phenylsuccinimide, Milontin, Parke Davis) was extracted from the commercially available capsules; naphthalene purity grade (internal standard) (BDH, Poole, U.K.); di-n-butyl phthalate puriss (international standard) (KEBO AB, Stockholm); chloroform and KH_2PO_4, reagent grades (Merck, Darmstadt, Germany).

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Extraction procedure

To 0·5 ml plasma or urine in a conical tube are added saturated KH_2PO_4 solution (0·1 ml; pH 5·4) and chloroform (0·2 ml) containing either naphthalene (3 μg) (internal standard for ethosuximide) or di-n-butyl phthalate (20 μg) (internal standard for phensuximide). The tube is shaken gently for 10 min and centrifuged at 1000 *g* for 20 min. One μl of the organic phase is then analysed by gas chromatography.

Gas chromatography

Quantitative determination of both drugs was with a Varian Model 1400 gas chromatograph with a glass column (1·8 m × 1·5 mm i.d.) packed with 3% OV–17 on Gas-Chrom Q, 80–100 mesh (Applied Science Inc., State College, Pa., U.S.A.). For the determination of ethosuximide the column temperature was 125° and for phensuximide it was 190°. The injector block was kept at 200° and the temperature of the flame ionization detector was 210°. Nitrogen, hydrogen and oxygen gas flow rates were 25, 25 and 250 ml min^{-1}. The concentrations of the drugs in the samples were calculated with the aid of calibration curves prepared by adding known amounts (10–200 μg ml^{-1}) of drugs to blank plasma or urine. The standard samples were run through the procedure and the ratio of the peak heights of compound and internal standard was plotted against known concentrations of the compound.

Patients

The method for ethosuximide was tested in samples from 10 hospitalized patients, aged from 9 to 15 years on continuous treatment with the drug. Doses were according to the clinical condition and were administered orally three times daily. A variety of other antiepileptic and sedative drugs was also being given to these patients.

Blood samples were drawn in heparinized tubes by venipuncture and centrifuged within 1 h. Plasma was separated and kept frozen until analysis (within 2 months).

In ten patients blood samples were drawn just before the morning dose and again 3 h later on each of 2–5 days. In 6 of the 10 patients blood samples were drawn just before morning dose and 2, 4 and 6 h thereafter.

No patients were available to test the method for phensuximide which is not commonly used in Sweden.

RESULTS AND DISCUSSION

The method has high capacity and is well suited for clinical use. Both ethosuximide and phensuximide can be measured with the same extraction procedure by varying the temperature of the column. Peaks obtained in the chromatograms are symmetrical and well defined for both drugs, and their internal standards (Fig. 1); calibration curves obtained in blank plasma and urine were linear in the range of 10–200 μg ml^{-1}.

Recovery was determined by measuring standard concentration of drug added to chloroform, plasma or urine. The recovery of ethosuximide in plasma is $48 \pm 3\%$ ($n = 7$) and urine $55 \pm 3\%$ ($n = 3$). Phensuximide is recovered to $77 \pm 1\%$ ($n = 8$) in plasma and to 100% in urine. The low extractibility of ethosuximide from plasma is due to its lower lipid solubility compared to phensuximide. The partition coefficient between chloroform and water is for ethosuximide 5·3 and for phensuximide, 1400.

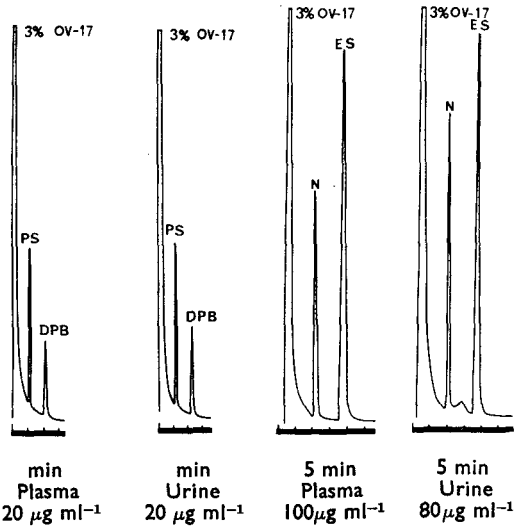

FIG. 1. Gas chromatograms of ethosuximide (ES) and phensuximide (PS). Naphthalene (N) and di-n-butylphthalate (DPB) were used as internal standards after extraction from human plasma and urine. Attenuation 2×10^{-10}.

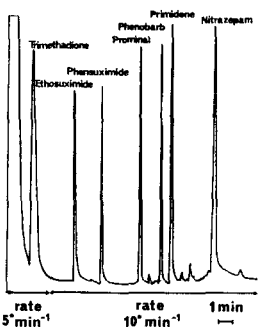

FIG. 2. Gas chromatogram after programming a sample between 100 and 250° showing the resolution of some anti-epileptic drugs currently in clinical use. Column 3% OV17 (80/100 mesh) length 6ft attenuation 4×10^{2}.

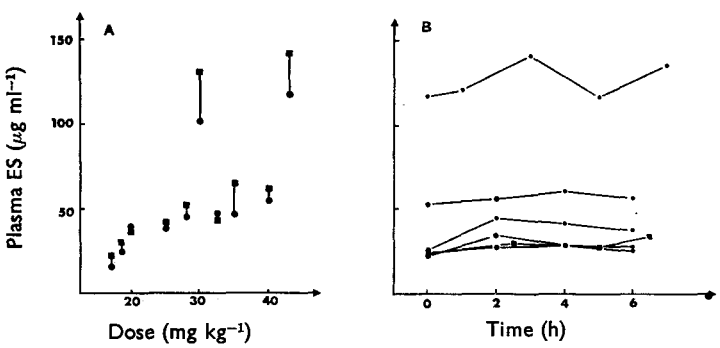

FIG. 3A. Plasma concentration of ethosuximide (μg ml^{-1}) before (●) and 3 h after morning administration (■) against dose (mg kg^{-1}), studied in 10 patients during chronic treatment. Every symbol is the mean of three to five samples taken on different days. The variation between different occasions in the same patient never exceeds 50%.

B. Plasma levels of ethosuximide against time (before dose, 2, 4 and 6 h after dose) in six patients chronically treated.

Reproducibility of the method was evaluated by analysing 28 samples from the same plasma pool containing known concentrations of ethosuximide. This gave a variation of $\pm$ 4%. The method is sensitive enough to measure plasma and urine levels of either drug down to 2·5 μg ml^{-1} (plasma concentrations of ethosuximide obtained clinically in our study varied between 10–150 μg ml^{-1}).

To check specificity we have analysed equimolar concentrations of other anti-epileptic drugs (Fig. 2), *i.e.* trimethadione, prominal, phenobarbitone, primidone and nitrazepam. No interference was observed in the chromatograms. The patients studied were concomitantly treated with a variety of other drugs but no interference was observed.

As the internal standard for phensuximide is butyl phthalate, it is pertinent to note that alkyl phthalates are commonly used as plasticizers in containers used for food products and have recently been found in the lipid extracts of human blood (Marcel, 1970). The possible occurrence of low amounts of n-butyl phthalate in blood plasma, thus, cannot be overlooked.

Plasma concentrations of ethosuximide

Fig. 3A shows the plasma levels of ethosuximide obtained in 10 patients before and 3 h after the morning dose. Each point is the mean of three to five samples taken on different days. The plasma concentrations at 3 h after morning dose is 24 $\pm$ 15% (n = 27) higher than the value obtained before the morning dose.

In six patients, plasma levels were measured every second hour during the 6 h dosage interval (Fig. 3B); minor variations were found.

In all but two of the patients, plasma concentrations varied between 10 and 60 μg ml^{-1} (at doses between 10 and 40 mg kg^{-1}). Two patients dosed at 30 and 45 mg kg^{-1} respectively had plasma concentrations between 100 and 150 μg ml^{-1}.

These data clearly indicate marked interindividual differences in the kinetics of ethosuximide. The plasma disappearance and urinary excretion of a single dose of ethosuximide was studied in one healthy volunteer. Approximately 20% was excreted unchanged within 60 h while the plasma half-life was 48 h suggesting that ethosuximide is metabolized to a considerable extent.

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Note added in proof: After this work had been completed Penry & others (1972) [in *Anti-epileptic Drugs*, Editors: Woodbury, D. M., Penry, J. K. & Schmidt, R. P., pp. 431–441. New York: Raven Press] reported that maximum clinical control with ethosuximide was achieved with plasma concentrations of from 40–80 μg ml^{-1}.

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